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Rootstock determines drought resistance of poplar grafting combinations to water deficit

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Head title: Rootstock determines drought resistance of poplar

Abstract To increase production and/or enhance drought resistance, grafting is often applied in

24 agriculture and horticulture. Interspecific grafting could probably be used in forestry as well to improve
25 drought resistance, but our understanding of how the rootstock of a more drought-resistant species can
26 affect the grafted plant is very limited. Reciprocal grafts of two poplar species, *Populus cathayana*
27 (less drought-resistant, C) and *P. deltoides* (more drought-resistant, D) were generated. Four grafting
28 combinations (scion/rootstock: C/C, C/D, D/D and D/C) were subjected to well-watered and drought-
29 stress treatments. C/D and D/C had a higher diameter growth rate (GR_D), leaf biomass, intrinsic water
30 use efficiency (WUE_i) and total non-structural carbohydrate (NSC) content than C/C and D/D in well-
31 watered condition. However, drought caused greater differences between *P. deltoides*-rooted and *P.*
32 *cathayana*-rooted grafting combinations, especially between C/D and D/C. C/D grafting combination
33 showed higher resistance to drought, as indicated by a higher stem growth rate, P_n , WUE_i , leaf water
34 potential, proline concentration and NSC concentration, and a better integrity of the leaf cellular
35 ultrastructure under drought when compared to D/C. D/C exhibited severely damaged cell membranes,
36 mitochondria and chloroplasts under drought. The scion genotype caused a strong effect on the root
37 proline concentration: *P. cathayana* scion increased the root proline concentration more than *P.*
38 *deltoides* scion (C/C vs D/C, C/D vs D/D) under water deficit. Our results demonstrated that the
39 rootstock determined the drought resistance of grafting combinations, although the scion may also
40 influence this resistance. Grafting of a *P. cathayana* scion onto a *P. deltoides* rootstock resulted in
41 superior growth and biomass when compared to the other three combinations both in well-watered and
42 drought stress conditions.

43 **Keywords:** poplar, grafting combination, scion genotype, water use efficiency, non-structural
44 carbohydrates

Introduction

The global climate change is expected to increase the probability of drought episodes and extreme climatic events (Saxe et al. 2001). Severe drought events have caused forest decline and mortality in many forests during recent decades (Michaelian et al. 2011, Luo and Chen 2015, Assal et al. 2016). Poplars are world-widely occurring, fast-growing trees with potential for high productivity, but they are very sensitive to water availability (Monclus et al. 2006). In particular, drought can strongly reduce productivity and it leads to increased mortality in poplar forests (Rehfeldt et al. 2009, Michaelian et al. 2011, Assal et al. 2016). While commercial poplar genotypes have been selected primarily based on criteria, such as high productivity (Monclus et al. 2006, Raj et al. 2011), responses and resistance of poplars to drought require more attention, especially considering the global change.

To increase production or to enhance resistance to abiotic stresses, grafting is commonly used in agriculture and horticulture (Vanderklein et al. 2007, Greenwood et al. 2010, Tombesi et al. 2010, Huang et al. 2016). Grafting combines genotypes that differ in traits like carbon fixation and translocation, water uptake and transport, which rely on shoot elongation originating from the scion and have a dominant effect on shoot biomass (Kita et al. 2018, Li et al. 2018). On the other hand, the uptake of belowground resources, such as soil water, relies on root growth that differs among rootstock genotypes (Alsina et al. 2011, Albacete et al. 2015, Zhang et al. 2016). Combining scion and rootstock provides opportunities to exploit genetic variation for specific root or shoot traits (Albacete et al. 2015). Grafting a scion onto a rootstock with a higher drought resistance can improve the stress resistance of the whole plant, because the rootstock modifies the xylem vessel diameter or density to increase the

67 hydraulic conductivity and to reduce the cavitation of the scion (Tombesi et al. 2010, Alsina et al. 2011,
68 Bauerle et al. 2011). Leaves are more vulnerable to drought compared to roots (Matsuo et al. 2009,
69 Dong et al. 2016), and root modifications strongly impact the physiological processes of leaves during
70 periods of drought (Han et al. 2013, Puértolas et al. 2017). The rootstock can enhance the resistance
71 of the scion to water deficit by increasing water use efficiency and storage of non-structural
72 carbohydrates as well as by controlling photosynthesis and stomatal conductance (Lovisolo et al. 2008,
73 Alsina et al. 2011, de Ollas et al. 2018, Han et al. 2018). The grafting technique has received attention
74 in forest tree breeding as well (Han et al. 2018, Kita et al. 2018). It is reasonable to assume that proper
75 grafting of poplars would enhance resistance to water deficit and increase wood productivity in poplar
76 plantations.

77

78 Plants with improved water use efficiency and increased water uptake perform in a superior way in
79 water-limited environments (Galmés et al. 2012, Flexas et al. 2013, 2016). Poplar species or genotypes
80 can enhance their drought tolerance by increasing the intrinsic water use efficiency of leaves (WUE_i)
81 and concentrations of proline or antioxidant enzymes (Xu et al. 2008, Han et al. 2018). Based on a
82 study including twenty-nine genotypes of *Populus deltoides* × *P. nigra*, Monclus (2006) concluded
83 that among the most productive genotypes many were drought-susceptible, while the less productive
84 genotypes displayed a large range of drought tolerance. By comparing the drought responses of six
85 poplar species, Cao et al. (2012) concluded that *P. cathayana* with the highest total stomatal density
86 and lowest WUE_i and carbon isotope composition ($\delta^{13}C$) was more susceptible to drought and
87 demanded more water than other species, including *P. deltoides*. In the present study, we examined
88 growth and physiological traits of reciprocal grafts between *P. cathayana* and *P. deltoides* with the aim

to find the optimal grafting combination(s) suitable for poplar plantations under drought stress. We hypothesized that grafting *P. cathayana* scion onto *P. deltoides* rootstock (C/D) will result in a graft combination that is more resistant to water deficit than other graft combinations, including both inter- and intraspecific grafting patterns. Although scion phenotypes can be altered widely by rootstocks (Gautier et al. 2019), little attention has been paid on the impact of the scion on the rootstock phenotype.

Materials and methods

Plant material and experimental design

Cuttings of *P. cathayana* were collected from the experimental nursery garden of the Sichuan Academy of Forestry (Sichuan province, China, 30°67'N, 104°06'E). Cuttings of *P. deltoides* were collected from the experimental nursery garden of the Nanjing Forestry University (Jiangsu province, China,

111 32°03'N, 118°46'E). After sprouting, 120 healthy cuttings (60 cuttings of *P. cathayana* and 60 of *P.*
112 *deltooides*) of approximately the same size (length ca. 15 cm, 2-3 buds) were selected for grafting. The
113 splice grafting technique was used (details in Supplementary Fig. 1). Altogether, four grafting
114 combinations were created, including two intraspecific combinations, i.e., *P. cathayana* scion with *P.*
115 *cathayana* rootstock (C/C) and *P. deltooides* scion with *P. deltooides* rootstock (D/D), and two
116 interspecific combinations, i.e., *P. cathayana* scion with *P. deltooides* rootstock (C/D) and *P. deltooides*
117 scion with *P. cathayana* rootstock (D/C).

118

119 On 12 March 2016, the grafted plants were planted in 10-L plastic pots filled with homogenized soil.
120 The grafting compatibility between *P. cathayana* and *P. deltooides* was good, as the survival rate of all
121 grafted plants exceeded 95% and the plants grew well (Supplementary Fig. 2). After two months of
122 growth, the grafted plants were subjected to two treatments, well-watered (100% soil field capacity,
123 average soil water content 36.8%) and drought stress (30% soil field capacity, average soil water
124 content 11.5%), for three months until 12 August 2016 (Han et al. 2013; Chen et al. 2014). The
125 experimental design was completely randomized. Each watering regime included 60 individuals (15
126 individuals per grafting combination). To monitor the soil water dynamics, three pots selected from
127 each treatment were weighed every other day and then re-watered to the designated soil water content.
128 The soil water content was similar for all grafting combination in both well-watered and drought
129 treatment conditions, respectively (Supplementary Fig. 3).

130

131 *Plant morphology, growth and biomass measurements*

132

The height (H) and basal diameter (BD) of each plant were measured at the beginning and at the end of the experiment. The height growth rate (GR_H) and the diameter growth rate (GR_D) were calculated as in Han et al. (2018). Five grafted plants were chosen and measured for height and basal diameter from each treatment at the end of the experiment. Thereafter, all plants were harvested, dried at 80 °C to a constant mass and, then, their dry mass was determined.

Gas exchange measurements

Before harvesting, one fully expanded and sun-lit leaves (4th or 5th leaf counted from the top of the plant) from five selected plants per treatment were used for gas exchange measurements. Net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) were measured with a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) from 08:30 to 11:30 a.m. on 28 and 29 July 2016. A standard LI-COR leaf chamber (2 cm × 3 cm window area) was used. The standard environmental characteristics during the measurements were as follows: leaf temperature, 25 °C; relative air humidity, 50%; ambient CO₂ concentration, $400 \pm 5 \mu\text{mol mol}^{-1}$; leaf-to-air vapor pressure deficit, $1.5 \pm 0.5 \text{ kPa}$; and photosynthetic photon flux density PPFD, $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Prior to the measurements, each selected leaf was illuminated with $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ provided by the LED light source of LI-6400 for 10 min to achieve full photosynthetic induction. Once the steady-state gas exchange rates were observed under these conditions, the steady-state data were recorded. Intrinsic water use efficiency (WUE_i) was calculated as the ratio of P_n to g_s (Zhang et al. 2012; Flexas et al. 2016).

Measurements of proline and non-structural carbohydrate (NSC) contents

On 12 August 2016, the stems, roots and leaves (from plants used to measure gas exchanges) were harvested, dried and ground. Leaves used for photosynthesis measurements were harvested to estimate the carbon isotope composition ($\delta^{13}\text{C}$). Proline was extracted and measured as described by Lei et al. (2006). Approximately 0.2 g of dry leaf powder was homogenized in 4 ml of 3% (w/v) aqueous sulfosalicylic acid solution. The homogenate was centrifuged at 3000 g for 10 min. A volume of 1 ml of the supernatant was transferred to a new tube and treated with 2 ml acid ninhydrin (a solution of 2.5 g ninhydrin/100 ml containing glacial acetic acid, distilled water, and 85% *o*-phosphoric acid at a ratio of 6:3:1) boiled for 60 min, and cooled down in a water bath at room temperature (25 °C). A volume of 2.5 ml of toluene was added, and after 30 s of shaking, two phases were separated. The content of proline (in the upper hydrophobic phase) was determined from absorbance at 520 nm (Multiskan Go-1510, Thermo Fisher Scientific Oy, Vantaa, Finland) and using L-proline as a standard. Five replicates were measured for each sample, and the proline contents were expressed as micrograms per gram ($\mu\text{g g}^{-1}$) of dry mass.

Total soluble sugars, fructose, sucrose and starch were estimated for each plant organ in five replicates from each treatment, as follows: 50 mg of fine-ground plant material in 4 ml of 80% (v/v) ethanol was placed in a water bath at 80 °C for 30 min, and centrifuged at 5000 g for 5 min. After that, the supernatant was transferred to a 10-ml centrifuge tube. The extraction was repeated twice with 2 ml of 80% (v/v) ethanol solution, and the supernatants were combined into a 10-ml centrifuge tube. Total soluble sugars were detected colorimetrically at 625 nm following the anthrone-sulfuric acid method

177 using glucose as a standard (Yemm and Willis 1954). Fructose and sucrose were detected
178 colorimetrically at 480 nm following the modified resorcinol method (Murata et al. 1968). Residues
179 left in the centrifuge tubes after sugar extraction were used to determine starch, which was detected
180 colorimetrically at 620 nm according to the anthrone-sulfuric acid method using glucose as a standard
181 (Dubois et al. 1956). The total starch and soluble sugar contents for entire plants were calculated
182 according to the methods of Jordan et al. (2014).

183

184 *Carbon isotope composition ($\delta^{13}\text{C}$) measurements*

185

186 Leaves used for photosynthesis measurements were harvested and ground into powder to estimate the
187 carbon isotope composition ($\delta^{13}\text{C}$). Three leaf samples were chosen from the five replicates per
188 treatment to determine $^{13}\text{C}/^{12}\text{C}$ ratios using an isotope ratio mass spectrometer (DELTA V Advantage,
189 Thermo Fisher Scientific, Inc., USA). The carbon isotope composition, $\delta^{13}\text{C}$, was expressed relative
190 to the standard Pee Dee Belemnite (Farquhar et al. 1989) as follows: $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$,
191 where R_{sample} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and R_{standard} that of the standard.

192

193 *Measurements of predawn leaf water potential (Ψ_{pd}) and relative water content (RWC)*

194

195 RWC and Ψ_{pd} were measured before the pots were rewatered. The 4th fully expanded leaf of each plant
196 (three replicates per treatment) used to monitor the water soil content was chosen to measure the
197 predawn leaf water potential (Ψ_{pd}) and relative water content (RWC). Ψ_{pd} was measured with a WP4
198 Dewpoint Potentiometer (Decagon Devices, Inc., Pullman, WA, USA). For each leaf, two replicate

199 measurements with 4 cm-diameter leaf discs were conducted. The discs were cut from the middle of a
200 leaf avoiding the midrib. RWC was measured according to the method of Gullo and Salleo (1992) by
201 calculating first the fresh mass (FM), turgid mass (TM) and dry mass (DM) for 10 leaf discs obtained
202 from the central portion of each leaf, and RWC was calculated as $RWC = 100 (FM - DM) / (TM - DM)$.

203

204 *Cellular ultrastructure*

205

206 One plant was randomly chosen from the three pots used to monitor water soil in each treatment. Then
207 leaf sections (2 mm in length) from the intercostal areas in the middle part of the 4th fully expanded
208 leaf were selected. The sections were fixed in 2.5% (v/v) glutaral pentanedial in 0.2 M sodium
209 phosphate buffer (PBS, pH 7.0) for 3 h at 22 °C and post-fixed in 2% osmium tetroxide (OsO₄) for 2
210 h. Thereafter, the samples were sequentially dehydrated in 30%, 50%, 70% and 90% acetone and
211 embedded in Epon 812 for 2 h. Ultra-thin sections (80 nm) were sliced with an Ultracut E
212 ultramicrotome (Reichert-Jung, Austria), stained with both uranyl acetate and lead citrate, and
213 mounted on copper grids for viewing with an H-600IV TEM (Hitachi, Tokyo, Japan) at an accelerating
214 voltage of 60.0 kV.

215

216 *Statistical analyses*

217

218 All data were analyzed using the Statistical Package for the Social Sciences software (SPSS Inc.,
219 Chicago, IL, USA) version 17.0. Homogeneity of variances and normality of distributions were tested
220 for each variable before statistical analyses, and log-transformations were applied when needed. Three-

221 way ANOVAs were used to assess the effects of drought, rootstock, scion and their interactions.
222 Individual differences among means were tested using the Tukey' test after one-way ANOVA at a
223 significance level of $P < 0.05$. For WUE_i and stem fructose concentrations, we applied Tamhane's T2
224 *post hoc* comparisons, as their variances were still not homogeneous after data transformation. Mean
225 values and standard errors were determined for each variable. Principal component analysis (PCA)
226 with physiological traits was used to identify the most conspicuous effects of grafting type and drought
227 stress after standardizing the data. PCA analyses were performed using SIMCA-P14.1 (Umetrics AB,
228 Umeå, Sweden).

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235 **Results**

236

237 In the well-watered condition, plant individuals of the hetero-grafted combinations (C/D and D/C) had
238 a significantly higher basal diameter growth rate (GR_D), particularly C/D that had the highest growth
239 rates, and shoot and leaf biomass (Table 1, Fig. 1). Drought reduced growth rates and biomass of all
240 four grafting combinations (Table 1, Fig. 1, Supplementary Fig. 4). Under drought, C/D still showed
241 the highest growth rate and total biomass when compared to other grafting combinations, whereas
242 GR_H, GR_D and the total biomass of D/C suffered from a great decline compared to the well-watered

condition (reductions by 44.24%, 57.98% and 68.60%, respectively). RDM of *P. cathayana*-rooted grafting combinations (C/C and D/C) was higher than that in *P. deltoides*-rooted grafting combinations (C/D and D/D) under well-watered condition, but there were no significant differences between them under drought stress.

In the well-watered condition, C/D had significantly higher P_n , E and WUE_i compared to C/C, while D/C showed higher P_n and WUE_i than D/D (Fig. 2). Drought decreased P_n , g_s and E and led to increased WUE_i in all four combinations. *P. deltoides*-rooted grafting combinations, especially C/D, had higher P_n , g_s , E and WUE_i than other grafting combinations in the drought condition (Fig. 2). P_n and E were lowest in D/C under drought stress. The results indicate that the leaf gas exchange traits of D/C suffered severe damage relative to the C/D grafting combination.

RWC and Ψ_{pd} showed no significant differences among grafting combinations in the well-watered condition, whereas *P. deltoides*-rooted grafting combinations had higher leaf Ψ_{pd} under drought (Fig. 3a, b). All grafting combinations increased their water use efficiency, as indicated by increased $\delta^{13}C$, under drought. However, C/D and D/D had significantly greater $\delta^{13}C$ than C/C and D/C during water deficit (Fig. 3c). Both leaf and root proline concentrations of *P. deltoides*-rooted grafting combinations were much higher than those of *P. cathayana*-rooted grafting combinations (Fig. 4). When compared with plants in the well-watered condition, leaf proline concentrations of C/C, C/D, D/D and D/C increased under drought by 65.40%, 92.67%, 124.82% and 74.47%, respectively (Fig. 4a), while root proline concentrations of C/C, C/D, D/D and D/C increased by 97.61%, 177.30%, 177.68% and

264 69.67%, respectively (Fig. 4b). The higher values of Ψ_{pd} , $\delta^{13}C$ and proline concentrations in *P.*
265 *deltoides*-rooted grafting combinations indicated their superior drought resistance.

266

267 Drought, rootstock and their interactions significantly affected starch and fructose concentrations
268 (Supplementary Table 1). In the well-watered condition, root and stem starch concentrations of grafting
269 combinations showed no significant differences (Fig. 5a). By contrast, under drought, leaf starch
270 concentrations of C/C, C/D and D/C were significantly reduced, while stem starch concentrations of
271 C/C, C/D and D/D as well as root starch concentrations of C/D and D/D were significantly increased
272 (Fig. 5a). There was a tendency that the soluble sugar concentrations of leaves and stems increased
273 under drought, except for the leaf soluble sugars of D/C (Fig. 5b). Similarly, the leaf fructose
274 concentrations of C/C, C/D and D/D significantly increased, whereas the leaf sucrose concentration of
275 D/C significantly reduced under drought (Supplementary Fig. 5). NSC concentrations of all four
276 grafting combinations tended to be higher under drought than in the well-watered condition, except
277 for leaf NSC of D/C (Fig. 5c).

278

279 The *P. deltoides*-rooted grafting combinations C/D and D/D showed less decrease in leaf and root
280 starch contents, but a higher increase in the stem starch content compared to C/C and D/C. For example,
281 the stem and root starch contents of C/D increased by 71.65% and 11.98%, respectively, whereas those
282 of D/C declined by 60.16% and 57.20% under drought relative to the well-watered condition (Fig. 6d,
283 g). Similarly, the leaf, stem and total soluble sugar contents of D/C were significantly lower than those
284 in other grafting combinations under drought (Fig. 6b, e, k). The leaf, stem, root and total NSC contents
285 of D/C were the lowest among all treatments with 70.67%, 65.65%, 55.93% and 68.03% decreases,

286 respectively, under drought compared with those in the well-watered condition (Fig. 6c, f, i, l).

287

288 In the well-watered condition, the mesophyll cells showed smooth and continuous cell membranes and
289 the chloroplasts exhibited a typical structure with well-arranged thylakoid membranes in the granal
290 regions (Fig. 7). In the drought condition, leaves of D/C showed signs of premature leaf senescence,
291 including a greater degree of leaf yellowing, while other graft combinations showed fewer visual signs
292 of drought impact (Fig. 7). Under drought, the chloroplasts of C/C and especially those of D/C were
293 strongly swollen, with blurred grana lamellae, while their starch granules and nucleoli disappeared and
294 the number of plastoglobuli increased. D/C suffered from strongest drought effects: the cell wall and
295 membrane were twisted, the chloroplasts became swollen and did not attach to the wall, the
296 degradation of the mitochondria was severe, and mitochondrial cavities were visible. However, the *P.*
297 *deltoides*-rooted grafting combinations could protect leaf cells from drought damage.

298

299 PCA showed a clear separation based on the traits of the four grafting combinations under different
300 watering regimes (Supplementary Table 2; Fig. 8). In the well-watered condition, C/C and D/D were
301 well-separated from C/D and D/C (Fig. 8), indicating that there were significant differences between
302 homo- and hetero-grafting combinations. Furthermore, all grafting combinations were very well
303 separated from each other under drought, demonstrating that the sensitivity and responses to drought
304 stress were different among all four grafting combinations.

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323 **Discussion**

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325 The rootstock genotype plays a crucial role in drought tolerance, since the hydraulic conductivity of
326 roots depends on the rootstock (Dodd et al. 2009, Albacete et al. 2015, de Ollas et al. 2018, Han et al.
327 2018). Previous studies have revealed a positive relationship between the hydraulic conductance of
328 roots and scion growth (reviewed by Zhang et al. 2016). Higher growth rates of *P. deltoides* rootstock
329 grafts implied that they probably had a higher hydraulic conductivity and also a better water uptake

330 and water use efficiency. Rootstock traits, like a greater root length, contribute positively to stomatal
 331 conductance and transpiration of leaves in grafted grapevine under drought (Peccoux et al. 2018).
 332 Stomatal conductance (g_s), transpiration rate (E) and net photosynthetic rate (P_n) of *P. deltooides*
 333 rootstock combinations were higher than those in *P. cathayana* rootstock grafted plants, particularly in
 334 C/D compared to D/C under drought. Although there were no differences in root biomass under
 335 drought, the root biomass of C/C and D/C declined by 73.45% and 78.87%, respectively, and those of
 336 C/D and D/D by 66.31% and 70.52%, respectively, (Table 1). We also measured Ψ_{pd} before
 337 replenishing the pot water loss and, similarly, discovered that C/D and D/D showed higher Ψ_{pd} than
 338 C/C and D/C (Fig. 3b), which indicated less water loss in C/D and D/D. WUE_i is generally used to
 339 characterize instantaneous water use efficiency and $\delta^{13}C$ is used to indicate integrated water use
 340 efficiency (Monclus et al. 2006, Cao et al. 2012, Yu et al. 2019). WUE_i is positively correlated with
 341 $\delta^{13}C$ in poplar leaves (Cao et al. 2012). Higher WUE_i and $\delta^{13}C$ of C/D and D/D compared to those of
 342 C/C and D/C suggested that the *P. deltooides*-rootstock could maintain higher short- and long-term
 343 water use efficiency in scions under drought. The results demonstrated that *P. deltooides*-rooted plants
 344 can absorb more soil water at a given root biomass or that they can control leaf water loss more
 345 efficiently.

346

347 Proline has diverse roles under water deficit conditions, such as stabilization of proteins, membranes
 348 and subcellular structures, and protection of cellular functions by scavenging reactive oxygen species
 349 (van Rensburg et al. 1993, Sivakumar et al. 2000, Szabados and Savouré 2010). The accumulation of
 350 proline can drive water influx or reduce the efflux (an increase in cellular osmolarity) that contributes
 351 to a higher water potential and water use efficiency, and finally protects photosynthetic activities under

352 stress (Kishor et al. 2005). The proline content also correlates positively with membrane integrity,
353 because proline can protect thylakoid membranes against environmental stress (van Rensburg et al.
354 1993, Sivakumar et al. 2000). We discovered that drought significantly increased proline
355 concentrations in all grafting combinations, while proline concentrations in the leaves and roots of C/D
356 and D/D were significantly higher than those of C/C and D/C. The severity of cell membrane distortion,
357 chloroplast swelling and mitochondrial degradation could be explained by the proline concentration,
358 which was lowest in D/C under drought. The scions grafted onto the *P. deltoides* rootstock, particularly
359 C/D, kept relatively high P_n , E and g_s , and the chloroplasts were less harmed (slightly swollen, granum
360 lamellae clearly visible) under drought.

361

362 Under drought conditions, plants with higher levels of non-structural carbohydrates (NSC, the sum of
363 starch and soluble sugars) in leaves, stems or roots have a greater capacity to resist drought impacts
364 (Wiley et al. 2013, O'Brien et al. 2014). The primary function of NSC is energy storage, but they also
365 play an important role in osmotic adjustment and long-distance transport of energy from leaves to
366 heterotrophic organs (Hartmann and Trumbore 2016). The concentration of leaf soluble sugars is
367 positively related to Ψ_{pd} under drought, because soluble sugars, such as sorbitol, fructose and sucrose,
368 contribute to the maintenance of cell turgor (Adams et al. 2013, Simard et al. 2013). It has been
369 suggested that soluble sugars enhance the accumulation of proline and affect the drought resistance of
370 plants (Kishor et al. 2005). Starch is an important reserve to buffer environmental changes and to
371 supply energy to enhance survival under long-term drought periods (Niinemets 2010, Wiley et al. 2013,
372 Guo et al. 2016, Hesse et al. 2019). An inverse relationship between leaf starch and soluble sugar
373 contents has been reported in plants exposed to drought (Adams et al. 2013). The concentration of leaf

soluble sugars is promoted by drought, except for D/C, while the concentration of leaf starch decreases under drought, visible also as a disappearance of starch granules in chloroplasts (Fig. 5, 7, Guo et al. 2018). To survive during water deficit, plants sacrifice growth and accumulate NSC to sink organs (Jacquet et al. 2014). The stem starch concentration remarkably increased and the stem starch content tended to increase, although stem growth declined under drought (Fig. 5, 6). The higher accumulation of NSC in plants enhances their ability to survive better under drought (Wiley et al. 2013, Jacquet et al. 2014, O'Brien et al. 2014). *P. deltooides*-rooted graft combinations could keep sustainable carbon assimilation with higher P_n , while their higher leaf water potential contributed to photosynthate translocation during water deficit (see also Hesse et al. 2019). This together with the higher proline concentration can explain the higher drought resistance of C/D.

384

We hypothesized that the drought tolerance of the rootstock species is the key factor affecting the drought tolerance of the grafting combination; for instance, C/D is more drought-resistant than D/C, because *P. cathayana* is more vulnerable to drought than *P. deltooides*. Our results broadly supported the hypothesis that drought resistance of grafted plants depends on the rootstock. Growth rates, photosynthesis, WUE_i , and proline and NSC contents of C/D were much higher, whereas those of D/C declined more during drought. The leaf ultrastructure of D/C suffered greater damage under water deficit compared with C/D (Fig. 7). The distant position between D/C and C/D in the PCA analysis (Fig. 8) also implied their major difference in drought resistance. The higher drought resistance of *P. deltooides* rootstock with higher proline and NSC concentrations is advantageous for plants, allowing the maintenance of cell turgor and water uptake (Subbarao et al. 2000), water use efficiency as well as carbon fixation, carbon translocation, storage and growth (Galiano et al. 2017) under water deficit.

397 The scion traits are also important factors that impact the growth and performance of grafted plants.
398 Besides supplying photosynthates for root germination and growth, previous studies have reported the
399 role of shoot-borne signals in regulating root development (reviewed by Ko and Helariutta 2017).
400 Some studies have paid attention to the impact of scion on rootstock. White and Castillo (1989) have
401 suggested that the scion has no effect on the seed yield of common bean (*Phaseolus vulgaris*) and that
402 no scion and root interaction effect occurred. By contrast, when investigating reciprocal grafting
403 between wide-type (WT) tomato *Solanum lycopersicum* and ABA-deficient *flacca* (*flc*) tomato, Li et
404 al. (2018) found that the WT scion increased the root biomass, root water potential and root ABA
405 concentration of the *flc* rootstock, while the *flc* scion decreased those of the WT rootstock, which
406 demonstrated a dominant effect by the scion genotype on the root biomass. In our study, C/C and D/C,
407 as well as D/D and C/D were well separated from each other according to PCA, thus indicating scion
408 effects on grafting combinations. However, the phenotype of grafted plants is complex, because it
409 combines two different genotypes and causes scion \times stock \times environment interactions. Tandonnet et
410 al. (2010) have suggested that in grafted grapevine the scion genotype controls biomass allocation and
411 root development, thus providing proof for conferred root vigor by scion. We observed that the *P.*
412 *deltoides* scion significantly decreased the growth of the *P. cathayana* rootstock only under well-
413 watered conditions but had no obvious impact on the *P. deltoides* rootstock biomass, which suggested
414 that the scion effects on rootstock development largely depend on the soil water status as well as on
415 their interactions. The present study revealed that the *P. cathayana* scion increased the root proline
416 concentration more than the *P. deltoides* scion (C/C vs D/C, C/D vs D/D) under water deficit (Fig. 4b).
417 This implied that also the proper scion selection may affect drought resistance in grafted plants.

418 Although *P. deltooides* is more drought resistant than *P. cathayana* (Cao et al. 2012), the *P. deltooides*
419 scion did not increase the drought resistance of D/C compared with C/C.

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430 **Conclusions**

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432 This study showed that the drought resistance of *P. deltooides*-rooted grafting combinations is greater
433 than that of *P. cathayana*-rooted grafting combinations. Scion also affected the drought resistance of
434 grafted plants by influencing root development and physiological traits of the rootstock. However, it
435 was proved that the drought resistance of grafted plants depend on the rootstock rather than on the
436 scion. Grafting a *P. cathayana* scion onto a *P. deltooides* rootstock was the best grafting combination,
437 because it has superior growth and biomass compared to the other three combinations both in well-
438 watered and drought stress conditions. Our study suggests that the grafting technique can be potentially
439 applied to forestry. Selecting drought-resistant rootstock would give plants an advantage to resist

drought impacts.

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Conflict of interest The authors declare that they have no conflict of interest.

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663 **Table 1** Root dry mass (RDM), stem dry mass (SDM), leaf dry mass (LDM) and total dry mass (TDM)
664 in four grafting combinations under well-watered and drought conditions.

| Soil field capacity | Scion/Rootstock | RDM (g) | SDM (g) | LDM (g) | TDW (g) |
|---------------------|--------------------------|---------------|----------------|----------------|-----------------|
| 100% | C/C | 5.01 ± 0.09 d | 9.18 ± 0.45 c | 8.89 ± 0.24 de | 23.08 ± 0.79 d |
| | C/D | 3.77 ± 0.11 b | 12.72 ± 0.63 d | 10.19 ± 0.44 f | 26.68 ± 1.09 de |
| | D/D | 4.07 ± 1.11 b | 10.33 ± 0.51c | 7.76 ± 0.25 d | 22.12 ± 0.69 d |
| | D/C | 4.62 ± 0.06 c | 10.51 ± 0.34 c | 9.25 ± 0.30 ef | 24.38 ± 0.46 de |
| 30% | C/C | 1.33 ± 0.05 a | 4.99 ± 0.08 b | 4.16 ± 0.17 ab | 10.28 ± 0.37 b |
| | C/D | 1.27 ± 0.04 a | 6.59 ± 0.26 b | 6.44 ± 0.20 c | 14.10 ± 0.33 c |
| | D/D | 1.20 ± 0.08 a | 5.42 ± 0.20 b | 4.95 ± 0.12 b | 11.57 ± 0.25 b |
| | D/C | 1.41 ± 0.06 a | 2.99 ± 0.16 a | 3.25 ± 0.26 a | 7.66 ± 0.44 a |
| | Scion | 0.535 | <0.001 | <0.001 | <0.001 |
| | Rootstock | <0.001 | <0.001 | <0.001 | <0.001 |
| | Drought | <0.001 | <0.001 | <0.001 | <0.001 |
| | Scion×rootstock | 0.036 | 0.855 | <0.001 | 0.163 |
| | Scion×drought | 0.535 | <0.001 | 0.672 | 0.001 |
| | Rootstock×drought | <0.001 | <0.001 | <0.001 | <0.001 |
| | Scion×rootstock× drought | 0.001 | <0.001 | 0.006 | 0.001 |

665 *P* values of three-way ANOVAs were shown to reflect the effect of scion, rootstock, drought and their
666 interactions on RDM, SDM, LDM and TDM. Values are means ± standard error (*n* = 5). Different
667 letters indicate significant differences at *P* < 0.05 according to Tukey's tests.

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679 **Figure legends**

680 **Figure 1** Height growth rate (a) and stem diameter growth rate (b) in four poplar grafting combinations
681 under well-watered and drought conditions. C, *Populus cathayana*; D, *P. deltoides*. C/C and D/D
682 represent intraspecific grafting combinations of *Populus cathayana* and *P. deltoides*, respectively. C/D
683 and D/C represent interspecific grafting combinations of the two species. S: scion effect; R: rootstock
684 effect; D: drought effect; S×R: interaction effect of scion and rootstock; S×D: interaction effect of
685 scion and drought; R×D: interaction effect of rootstock and drought; S×R×D: interaction effect of scion,
686 rootstock and drought. Values are means ± standard error ($n = 5$). Different letters indicate significant
687 differences at $P < 0.05$ according to Tukey's tests.

688 **Figure 2** Net photosynthesis rate P_n (a), transpiration rate E (b), stomatal conductance g_s to water
689 vapor (c) and intrinsic water use efficiency WUE_i (d) in four poplar grafting combinations under well-
690 watered and drought conditions. The WUE_i values were compared by Tamhane's T2 *post hoc* test, as
691 variances of this trait were still not homogeneous after data transformation. Treatment and grafting
692 codes as in Figure 1. Values are means ± standard error ($n = 5$). Different letters indicate significant
693 differences at $P < 0.05$ according to Tukey's tests.

694 **Figure 3** Leaf relative water content RWC (a), predawn leaf water potential Ψ_{pd} (b) and leaf carbon
695 isotope composition $\delta^{13}C$ (c) in four poplar grafting combinations under well-watered and drought
696 conditions. Treatment and grafting codes as in Figure 1. Values are means \pm standard error ($n = 3$).
697 Different letters indicate significant differences at $P < 0.05$ according to Tukey's tests.

698 **Figure 4** Proline concentrations of leaves (a) and roots (b) in four poplar grafting combinations under
699 well-watered and drought conditions. Treatment and grafting codes as in Figure 1. Values are means \pm
700 standard error ($n = 5$). Different letters indicate significant differences at $P < 0.05$ according to Tukey's
701 tests.

702 **Figure 5** Non-structural carbohydrate concentrations of leaves, stems and roots, including starch (a),
703 soluble sugars (b), and NSC (c) in four poplar grafting combinations under well-watered and drought
704 conditions. Treatment and grafting codes as in Figure 1. Values are means \pm standard error ($n = 5$).
705 Different letters indicate significant differences at $P < 0.05$ according to Tukey's tests.

706 **Figure 6** Leaf (a-c), stem (d-f), root (g-i) and total non-structural carbohydrate contents (j-l) in four
707 poplar grafting combinations under well-watered and drought conditions. Treatment and grafting codes
708 as in Figure 1. Values are means \pm standard error ($n = 5$). Different letters indicate significant
709 differences at $P < 0.05$ according to Tukey's tests.

710 **Figure 7** Leaf morphology and transmission electron micrographs of mesophyll cells in four different
711 grafting combinations under well-watered and drought conditions. C/C, *P. cathayana* scion with *P.*
712 *cathayana* rootstock; C/D, *P. cathayana* scion with *P. deltoides* rootstock; D/D, *P. deltoides* scion with
713 *P. deltoides* rootstock; D/C, *P. deltoides* scion and *P. cathayana* rootstock. CW, cell wall; SG, starch
714 granule; Ch, chloroplast; P, plastoglobulus; M, mitochondrion; N, nucleus; NU, nucleolus; Gr, granum.
715 Bar = 2 μm .

716 **Figure 8** Principal component analysis (PCA) based on studied traits of four grafting combinations
 717 (C/C, C/D, D/D, D/C, see Fig. 1 for code definitions) under well-watered and drought conditions.
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723 **Figure 1**

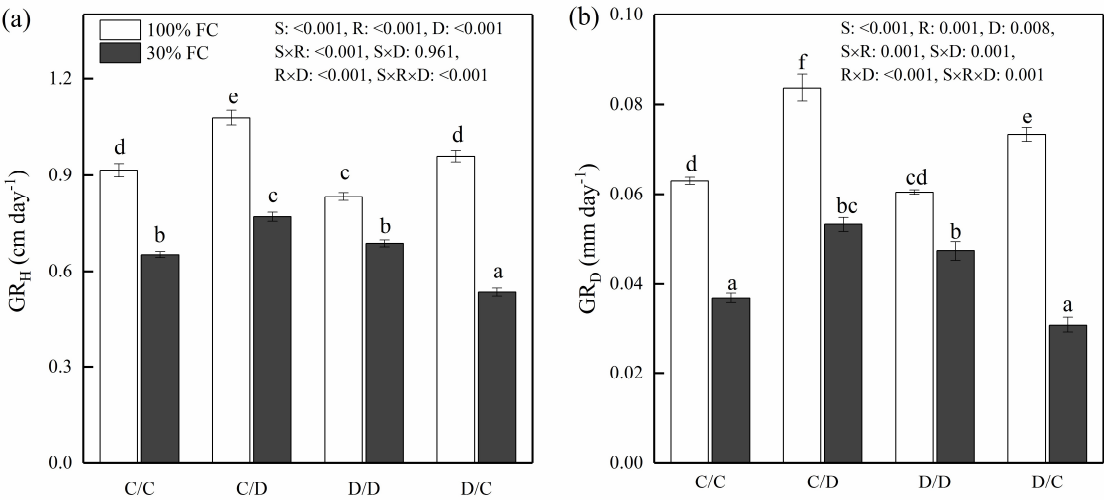
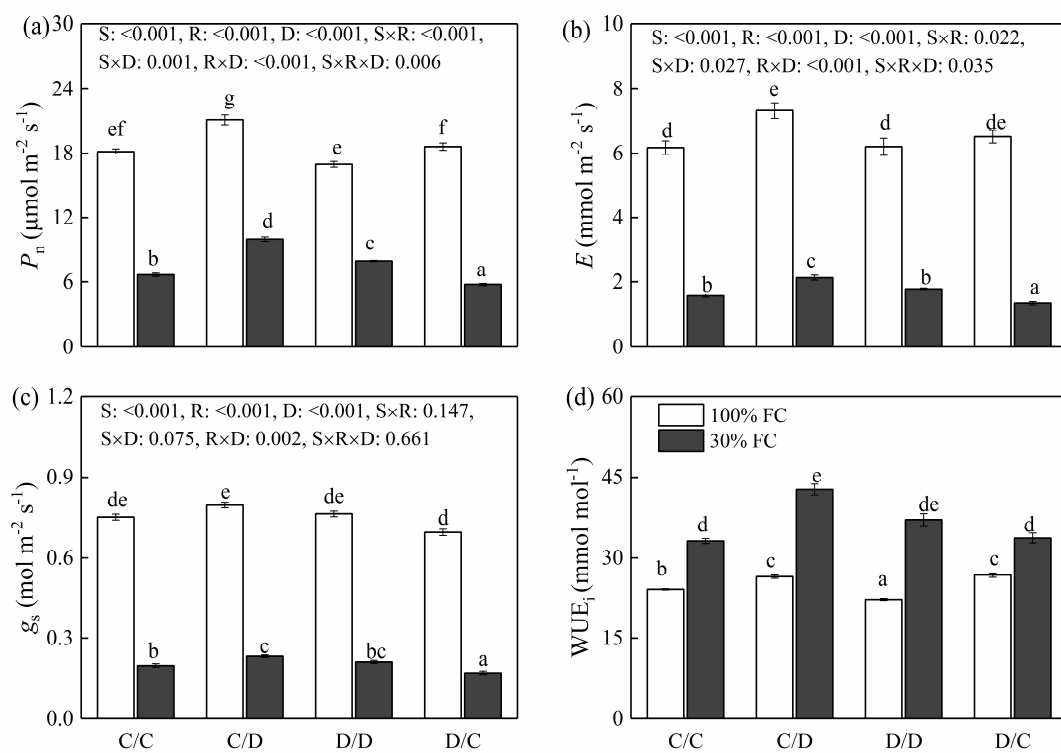


Figure 2



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Figure 3

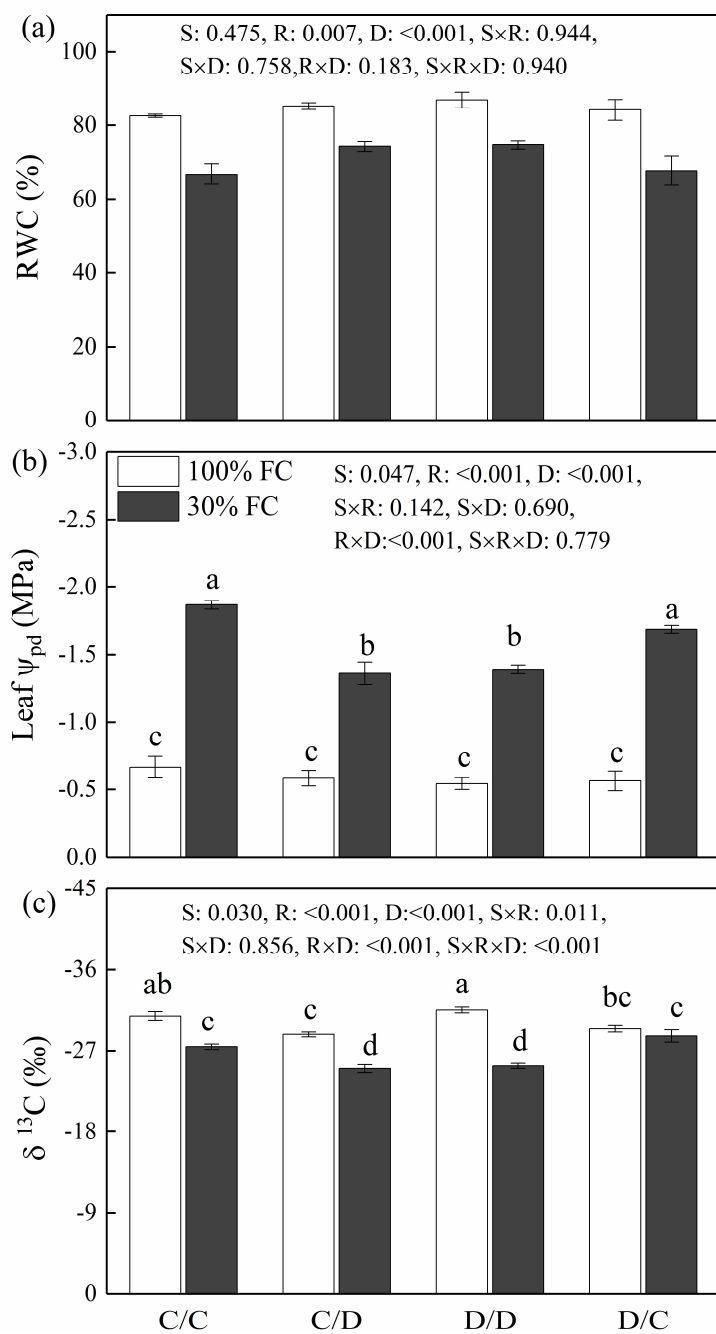


Figure 4

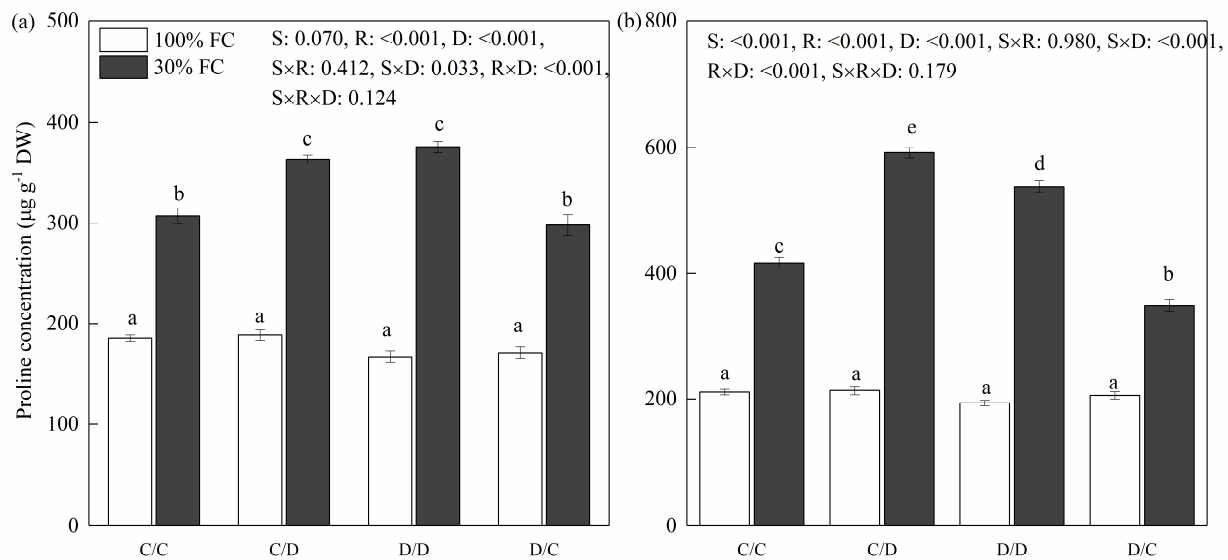
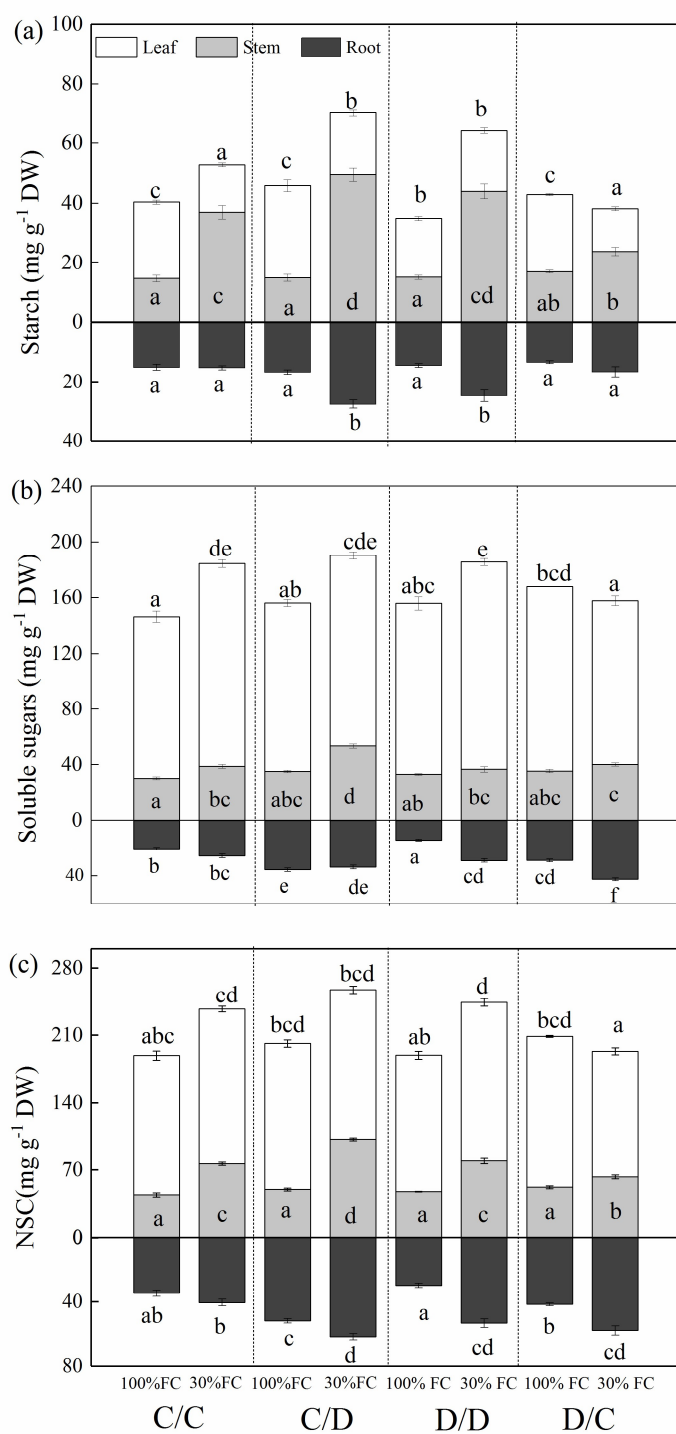
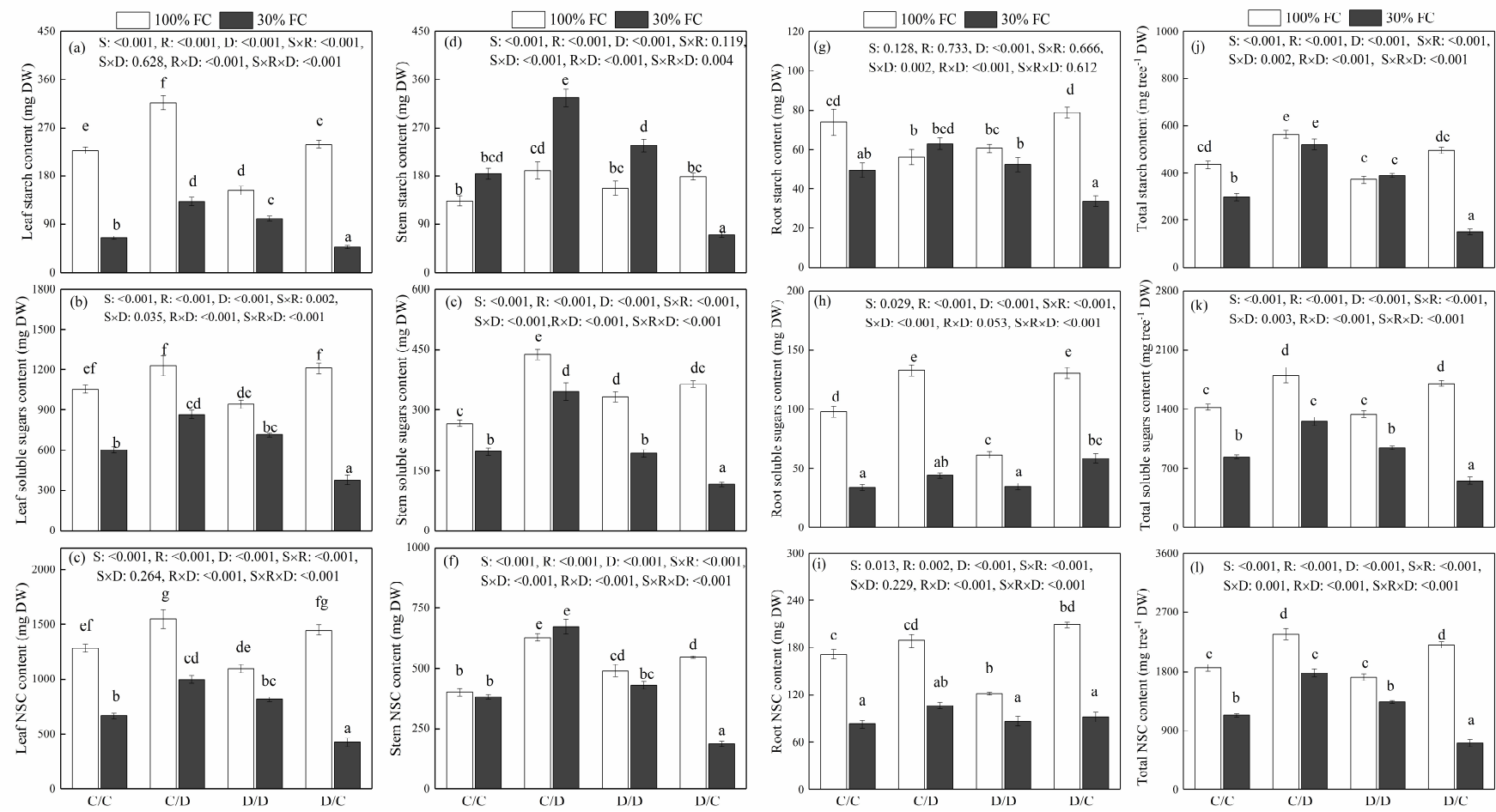
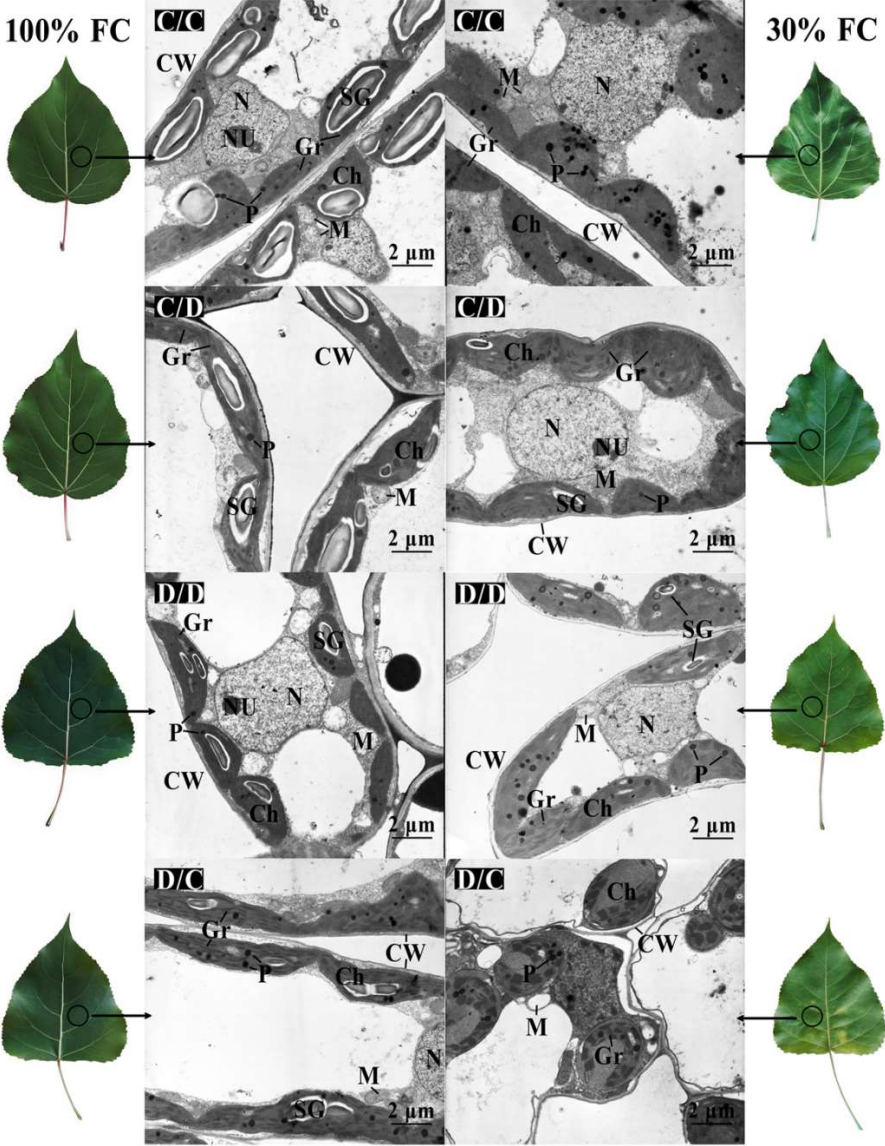


Figure 5

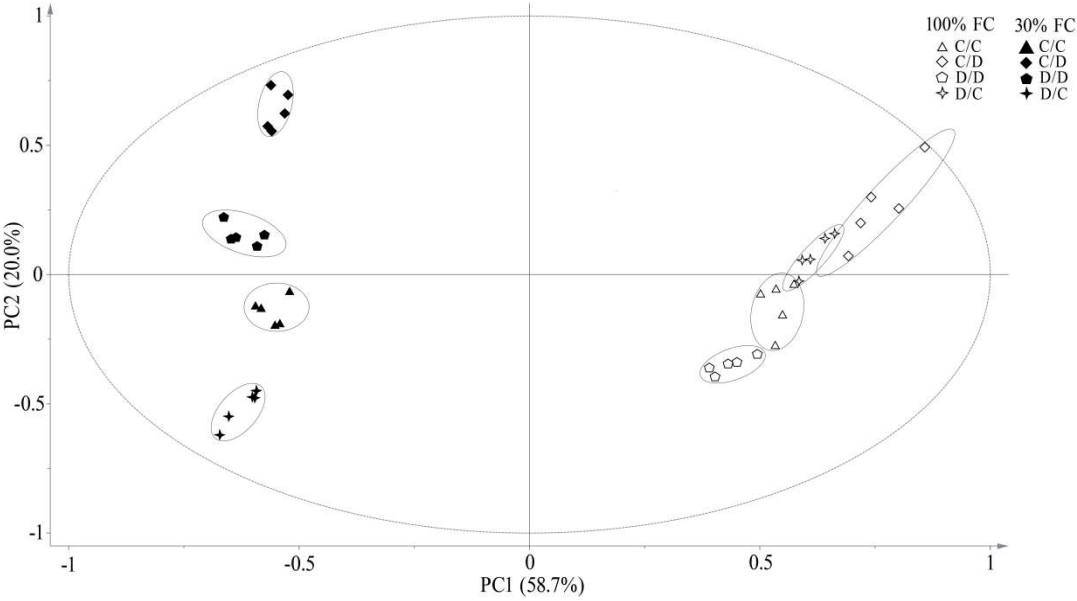




795 **Figure 7**



807 **Figure 8**



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